FEBS Letters 586 (2012) 3539–3547

journal homepage: www.FEBSLetters.org

Review

The FKBP families of higher plants: Exploring the structures and functions of protein interaction specialists

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ARTICLE INFO

Article history:
Received 7 August 2012
Revised 31 August 2012
Accepted 3 September 2012
Available online 13 September 2012

Edited by Michael R. Sussman

Keywords:
FK506-binding protein
PPIase
Immunophilin
Molecular chaperone
Plant
Stress response
Phosphorylation

ABSTRACT

The FK506-binding proteins (FKBPs) are known both as the receptors for immunosuppressant drugs and as prolyl isomerase (PPIase) enzymes that catalyse rotation of prolyl bonds. FKBPs are characterised by the inclusion of at least one FK506-binding domain (FKBd), the receptor site for proline and the active site for PPIase catalysis. The FKBPs form large and diverse families in most organisms, with the largest FKBP families occurring in higher plants. Plant FKBPs are molecular chaperones that interact with specific protein partners to regulate a diversity of cellular processes. Recent studies have found that plant FKBPs operate in intricate and coordinated mechanisms for regulating stress response and development processes, and discoveries of new interaction partners expand their cellular influences to gene expression and photosynthetic adaptations. This review presents an examination of the molecular and structural features and functional roles of the higher plant FKBP family within the context of these recent findings, and discusses the significance of domain conservation and variation for the development of a diverse, versatile and complex chaperone family.

1. Introduction

The FK506-binding proteins (FKBPs) were initially characterised as cellular targets for FK506 and rapamycin, macrolides produced by soil bacteria that are used as immunosuppressant drugs [1,2]. The FKBPs joined the unrelated cyclosporin-binding cyclophilin (CYP) proteins in the class of immunosuppressant receptors known as immunophilins [3]. In immune cells, the FK506–FKBP and cyclosporin–CYP complexes interact with the calcium-dependant phosphatase calcineurin (CaN), interrupting the phosphorylation signalling pathway required for expression of genes involved in immune response, while FKBP-bound rapamycin interacts with the kinase 'target of rapamycin' (TOR), causing cell cycle arrest that is also immunosuppressive [4]. Another characteristic of the FKBPs and CYPs is 'peptidyl-prolyl isomerase' (PPIase) activity; that is, the ability to catalyse rotation of the peptide bond immediately preceding a proline residue between *cis* and *trans* configurations [1,2,5]. The immunophilins share this property with parvulin, a non-immunophilin PPIase [6].

The FKBPs form large protein families in eukaryotes, the largest of which occur in higher plants, with 23 isoforms in *Arabidopsis thaliana* [7] and 29 *Oryza sativa* [8]. Since their initial exploration two decades ago [9], plant FKBPs have been implicated in a diversity of cellular processes including development, stress response, transcription regulation and chloroplast function (reviewed in [10,11]), and yet specific details of FKBP behaviour in plants have been difficult to pin down. Recent evidence indicates that plant FKBPs operate primarily as regulators of protein function through specific interactions with protein ligands, and that variation in FKBP sequence and structure has facilitated the evolution of a diverse family of molecular chaperones. In this review we examine the growing body of evidence shedding new light on the evolution and cellular functions of FKBP families in higher plants, and propose a general role for FKBPs in protein phosphorylation.

2. Domain compositions of plant FKBPs

2.1. The FK506-binding domain

FKBPs are characterised by the inclusion of at least one FK506-binding domain (FKBd), which is the receptor site for proline and proline analogues such as FK506 and rapamycin, and the active site for PPIase catalysis. The FKBd sequence of approximately 110 amino acids (Fig. 1) adopts a well-conserved tertiary structure [12–14], primarily containing six anti-parallel beta sheets connected by a number of solvent-exposed loops, one of which

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0014-5793/$36.00
http://dx.doi.org/10.1016/j.febslet.2012.09.002

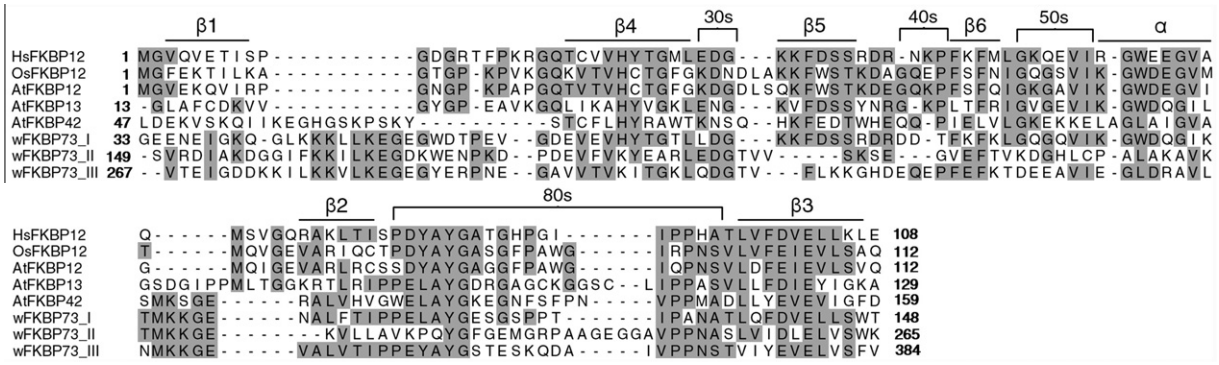

Fig. 1. Amino acid alignment of FK506-binding domains. Domains aligned are from FKBP isoforms of *Homo sapiens* (HsFKBP12; Genbank Accession NP_463460.1), *Oryza sativa* (OsFKBP12; LOC_Os06g27970), *Arabidopsis thaliana* (AtFKBP12; LOC_At5g64350, AtFKBP13; LOC_At5g45680, AtFKBP42; LOC_At3g21640) and wheat (wFKBP72 domains I, II and III taken from PDB accession 3JYM [72]). Bolded numbers indicate the position of the first and last residues in the original FKBP sequence. Bars span alpha helix (α) and beta sheet (β) regions of the tertiary structure of *H. sapiens* FKBP12 (PDB Accession 2PPN; [79]). Brackets show the loop regions as labelled. Shaded residues are identical, dashes indicate gaps in the alignment.

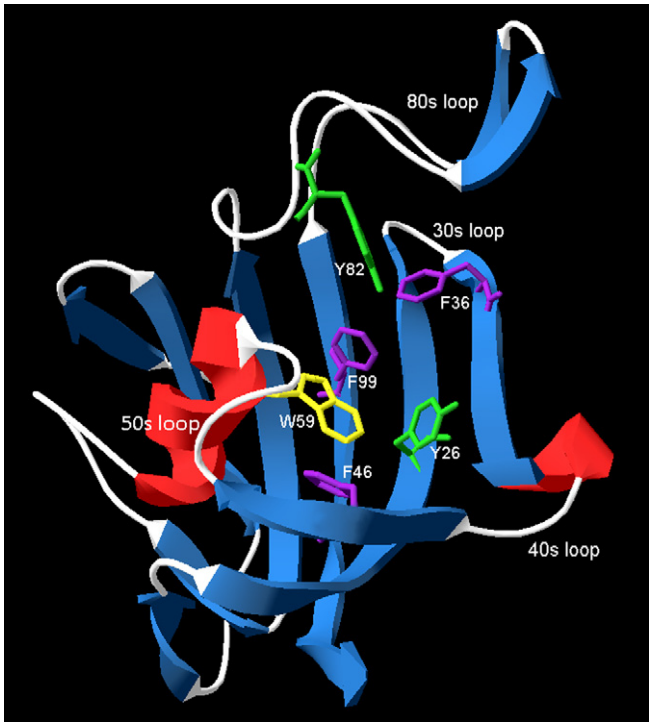

Fig. 2. The archetypal FK506-binding domain structure. Crystal structure of *H. sapiens* FKBP12 tertiary structure (PDB Accession 2PPN; [79]) showing beta strands (blue), helices (red) and loops (white), which are labelled in correspondence with Fig. 1. Five hydrophobic residues at the FKBd core are coloured and labelled according to their positions in HsFKBP12.

contains a short alpha helix (Fig. 2). The beta sheets form a concave surface opposite the helix and hydrophobic sidechains projected towards the protein core create a hydrophobic cavity that accommodates proline [15]. The loops, designated the 30s, 40s, 50s and 80s loops according to their location in archetypal FKBP sequence (Fig. 1), interact with substrates bound at the FKBP active site and also with secondary proteins bound at the FKBd periphery, such as CaN and TOR in mammals (reviewed in [16]). Despite strong conservation of the FKBd, a sizeable proportion do not bind drug ligands and lack any measurable PPIase activity (see Table 1), suggesting an alternative primary function for this domain that is discussed below.

2.2. Multidomain FKBPs

Plant FKBPs range in size from the 12 kDa isoform in Arabidopsis (AtFKBP12), comprising a single FKBd [17], to the 77 kDa isoform found in wheat (wFKBP77) [18]. Large FKBPs in plants and other species contain functional domains in addition to the obligatory FKBd, which is often repeated three times in tandem (Fig. 3 and Table 1). Also common are tetratricopeptide repeat (TPR) units that form anti-parallel alpha-helical domains, commonly providing the site for interaction with the large multipurpose heat shock chaperone HSP90 [19]. Calmodulin (CaM)-binding domains (CaMBds) frequently occur at the C-termini of large plant FKBPs and have been shown to actively bind the calcium sensor CaM [20,21]. The specific role of calcium signalling in the functioning of plant FKBPs has not been demonstrated, although CaM is a complex regulator of one mammalian FKBP that governs both HSP90 interaction at adjacent TPRs and substrate binding at the distal FKBd [22], suggesting similar calcium regulation may be possible for some multidomain FKPBs in plants.

3. Functional roles of plant FKBPs

3.1. Duplicated FKBPs in stress response

The multidomain isoforms FKBP62 and FKBP65, more commonly known as ROF1 and ROF2, respectively, share 85% identity in Arabidopsis [19,23] and comprise one of several sets of homologous FKBP duplicates found in plant genomes. Breiman and co-workers [24,25] discovered that ROF1 and ROF2 work antagonistically in the development of long-term tolerance to high temperature in Arabidopsis by modulating the expression of several small heat shock proteins (sHSPs) involved in recovery from heat stress. ROF1 binds via a TPR to HSP90, which in turn binds the heat shock transcription factor HsfA2 and the ROF1–HSP90–HsfA2 complex is then translocated to the nucleus where it induces expression of sHSPs and ROF2 [24]. ROF2 interrupts the nuclear ROF1–HSP90–HsfA2 complex by binding to ROF1 at its FKBd, thereby down-regulating sHSP expression during the recovery phase after heat shock [25]. Knockout of *rof1* severely compromised the plants' ability to cope with high temperature after a prolonged period of recovery (2–3 days) following initial heat shock, similar to the effect of *HsfA2* knockout, while *rof2* mutants and plants overexpressing ROF1 acquired better long-term heat tolerance than the wild type [24,25]. ROF2 was recently implicated in regulating intracellular pH and membrane polarity by controlling K^+ ion